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A substitution mutation in the myosin binding protein C gene in ragdoll hypertrophic cardiomyopathy

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Abstract

Familial hypertrophic cardiomyopathy (HCM) is a primary myocardial disease with a prevalence of 1 in 500 in human beings. Causative mutations have been identified in several sarcomeric genes, including the cardiac myosin binding protein C *(MYBPC3)* gene. Heritable HCM also exists in a large-animal model, the cat, and we have previously reported a mutation in the *MYBPC3* gene in the Maine coon breed. We now report a separate mutation in the *MYBPC3* gene in ragdoll cats with HCM. The mutation changes a conserved arginine to tryptophan and appears to alter the protein structure. The ragdoll is not related to the Maine coon and the mutation identified is in a domain different from that of the previously identified feline mutation. The identification of two separate mutations within this gene in unrelated breeds suggests that these mutations occurred independently rather than being passed on from a common founder.
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Hypertrophic cardiomyopathy is a primary myocardial disease characterized by increased left ventricular mass and wall thickness in the absence of a pressure overload or metabolic stimulus [1]. In human beings, it has a disease prevalence of 1 in 500 and is inherited, usually as an autosomal dominant trait, in at least 60% of the cases [1]. Causative mutations are most commonly single base pair substitutions found in one of several sarcomeric genes (β myosin heavy chain; cardiac myosin binding protein C; cardiac troponins T, I, and C; α tropomyosin; the essential and regulatory light chains; actin; and titin) [2–8].

Hypertrophic cardiomyopathy is the most common form of heart disease in the cat [9]. Affected cats are at risk of sudden cardiac death, development of congestive heart failure, and development of a systemic arterial thromboembolus. Increasingly, feline hypertrophic cardiomyopathy is noted to be present in specific breeds, with examples reported in the Maine coon, ragdoll, and British shorthair breeds, among others [9]. We have recently identified a causative mutation for inherited hypertrophic cardiomyopathy in the Maine coon cat in the gene for the cardiac myosin binding protein C *(MYBPC3)*, a sarcomeric gene and the second most common cause of familial hypertrophic cardiomyopathy in human beings [3,8,10,11]. The Ragdoll cat has also been reported to have a breed-specific form of hypertrophic cardiomyopathy. Some studies suggest a unique early onset form of the disease, with affected cats having an average age of diagnosis of 15 months compared to the reported age of diagnosis of 5–7 years for the general feline hypertrophic cardiomyopathy population [9,12].

The ragdoll breed is thought to be distantly related to the Persian cat breed and is not known to be related to the Maine coon cat breed [13]; therefore, it is unlikely to have a shared mutation passed down from a single founder. We hypothesized that this breed would have a unique mutation for hypertrophic cardiomyopathy, consistent with the allelic heterogeneity observed in human beings. The objective of this study was to evaluate the exonic and splice site regions of the cardiac myosin binding protein C gene in ragdoll cats with hypertrophic cardiomyopathy by PCR-based sequencing.

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Results and discussion

DNA samples from 21 ragdoll cats (10 female, 11 male) with a diagnosis of hypertrophic cardiomyopathy were submitted for evaluation. Age of diagnosis ranged from 9 months to 5 years. Eight of the cats were members of two families. In Family 1, both parents and three available offspring were known to be affected. In Family 2, both parents and one offspring were affected.

DNA sequencing revealed a single base pair change from a C to T in codon 820 in all of the affected cats (9 homozygotes, 11 heterozygotes) and none of the unaffected control cats. This change in the first base pair of the codon altered a conserved amino acid from arginine, a basic, positively charged amino acid, to tryptophan, a neutral, aromatic, hydrophobic amino acid (Table 1). No other mutations in other exonic or splice site regions of the *MYBPC3* gene were identified in affected ragdoll cats. The previously reported Maine coon mutation in exon 3 of *MYBPC3* (A31P) was not identified in any of the affected ragdoll cats [11].

The potential impact of this amino acid substitution was evaluated with PolyPhen (http://tux.embl-heidelberg.de/ramensky/polyphen.cgi). The substitution was predicted to be "probably damaging," with a PSIC score difference of 2.552, which implies a high confidence that the substitution affects protein function or structure. Protein structure analysis programs predicted a disruption of the α-helix structure and an increase in extended strand within the domain containing the mutation. The GOR4 program predicted that in the region of interest in the normal cat, 44% will be an α-helix structure, 9% in an extended strand, and 47% in a random coil. Evaluation of the same region containing the amino acid substitution predicted that the region would be 40% α helix, 17% extended strand, and 43% random coil. The Protean program also predicted an increase in the hydrophobicity in this region of the molecule in the affected cats (Fig. 1).

Age of diagnosis was available for all 9 of the cats homozygous for the mutation and ranged from 4 to 48 months (mean of 21 months). The age of diagnosis was available for 11 of the cats heterozygous for the mutation and ranged from 9 to 65 months (mean of 39 months). The age of diagnosis between the heterozygous and the homozygous cats was not significantly different.

Table 1
Partial amino acid sequence of the MYBPC3 transcript across species

Human	YILERKKKKSYRWM**R**LNFDLLRELSHEARRMIEGV
Mouse	YILERKKKKSYRWM**R**LNFDLLRELSHEARRMIEGV
Rat	YILERKKKKSYRWM**R**LNFDLLRELSHEARRMIEGV
Dog	YILERKKKKSYRWM**R**LNFDLLRELSHEARRMIEGV
Cow	YILERKKKKSYRWM**R**LNFDLLRELSHEARRMIEGV
Cat, normal	YILERKKKKSFRWM**R**LNFDLLQELSHEARRMIEGV
Cat, mutation	YILERKKKKSFRWM**W**LNFDLLQELSHEARRMIEGV

Partial amino acid sequence of the MYBPC3 transcript demonstrates the conservation of the arginine amino acid (R820) across species (GenBank Accession Nos. AY5183901, CAM17567L, XP_21577, DQ23807.1, BC119825.1). The ragdoll cardiomyopathy mutation changes the first base pair of the codon, which alters the amino acid to tryptophan (R820W).

In this report, we have identified the second mutation known to cause an inherited form of hypertrophic cardiomyopathy in the domestic cat. This newly identified mutation in the ragdoll cat was found in the myosin binding protein C gene, the same gene as the previously reported mutation in the Maine coon cat breed but in a different region of the gene [11]. In the Maine coon cat the amino acid affected (A31P) was located in the linker region between domains C0 and C1 of the protein. The functional aspects of that area are not well understood, but there is some evidence that domain C0 and the C0–C1 linker region may bind to myosin and/or actin [14–17]. The mutation reported in ragdoll cats is located in domain 6, a fibronectin type III region. Causative mutations for the human form of the disease have been observed within this domain as well, including a 3-bp deletion that affects the same codon as the one containing the mutation we describe [8,18–21]. A specific function for domain 6 has not yet been identified, although it has been suggested that it may interact with domain 9 to form overlapping regions that provide a collar around the sarcomeric myosin heavy chain thick filament [14,22]. This theory is supported by a previous study proposing interactions of domains 5 and 8, as well as 7 and 10 [22]. If this is the case, the amino acid change from arginine, a simple basic amino acid, to tryptophan, a very large aromatic amino acid, and the subsequent proposed change in secondary structure may interfere with these domain interactions.

Hypertrophic cardiomyopathy in the ragdoll cat has been previously characterized as an early onset form of the feline disease [12]. In one study, affected cats had an average age of diagnosis of 15 months (range, 5–24 months) compared to the reported age of onset of 4.8–7 years for the general feline hypertrophic cardiomyopathy population [9,12]. Although the study described here was not designed to evaluate closely for genotypic to phenotypic correlations it may be of interest that the cats that were homozygous for the mutation appeared to develop the disease, on average, 18 months earlier than the heterozygotes. This is consistent with our previous report of the *MYBPC3* mutation in Maine coon cats, for which the clinical outcome of the disease also varied with genotype. In that study, a larger number of cats homozygous for the mutation developed moderate to severe disease and most died of their disease at 4 years of age or less, whereas 3 of 10 Maine coon cats heterozygous for the mutation were still alive at 8–12 years of age, with moderate disease [11]. Two human studies have also suggested a role for gene dosage on the phenotype of hypertrophic cardiomyopathy mutations, including examples in the *MYBPC3* gene [8,19]. In one study, the same mutation at codon 810 was observed in two unrelated human patients. One individual was homozygous for the mutation and had severe left ventricular hypertrophy, while the other individual was heterozygous for the mutation and demonstrated only moderate nonobstructive hypertrophy [19].

In this study, we describe the second mutation associated with feline hypertrophic cardiomyopathy. Although this mutation is in the same gene as the previously reported feline mutation, its location is in a domain distant from the previously

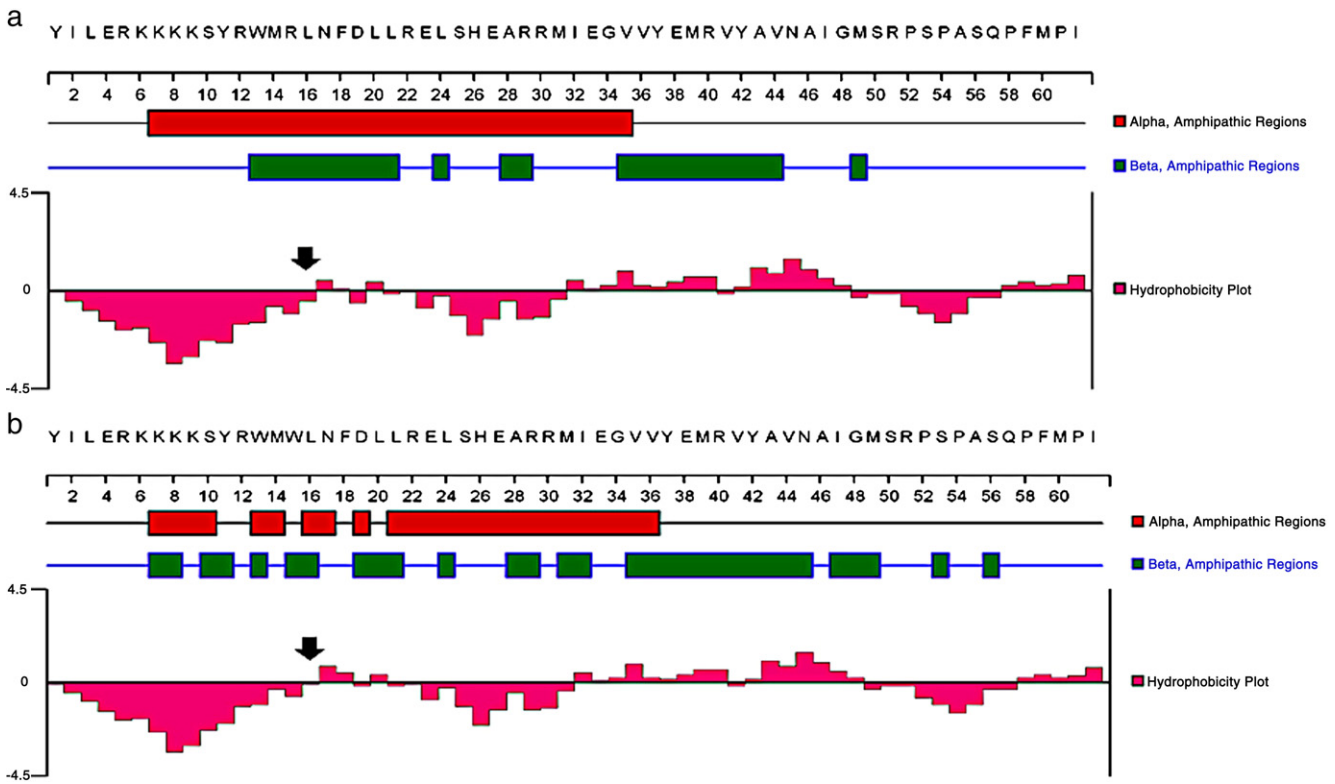

Fig. 1. Secondary structure analysis predicted that the change from (a) arginine to (b) tryptophan would result in a disruption of the α-helix and β-sheet structure and increased hydrophobicity (arrow denotes amino acid region, more hydrophobic above the line, less hydrophobic below the line) of the molecule in the mutated region.

reported one. Since this appears to be a private mutation for the ragdoll cat, the results reported here suggest that this mutation developed independently in the ragdoll cat breed, as opposed to a founder effect mutation brought in from a shared founder in one breed. The identification of two separate feline *MYBPC3* hypertrophic cardiomyopathy mutations in these two different breeds is consistent with the allelic heterogeneity observed in the human disease [8].

Numerous examples of both allelic and genetic heterogeneity have been identified within sarcomeric genes in human beings with HCM [2–8]. The results of this feline study demonstrate that allelic heterogeneity also exists in the cat with hypertrophic cardiomyopathy. Additional studies to evaluate the development of mutations within this gene as well as the effect of this specific mutation on the c-MyBP-C protein interactions and function are needed.

Materials and methods

DNA samples were obtained from 21 affected ragdoll cats previously diagnosed with hypertrophic cardiomyopathy by identification of left ventricular wall thicknesses exceeding the normal feline values and, sometimes, papillary muscle hypertrophy, as determined by echocardiography [9]. DNA samples were also obtained from 110 unaffected cats (controls) from a variety of different breeds, including ragdolls (19), sphinx (6), Maine coon (12), domestic shorthair (10), pixie bob (4), Bengal (4), Scottish fold (7), Persian (2), and rex (4), as well as mixed-breed cats (42).

The sequences of the exonic and splice site regions of the feline *MYBPC3* gene were extracted from feline contigs using the Blast function of NCBI and the human sequence (GenBank Accession No. U91629). Amplification primers were designed for each of the 35 exons using Primer 3 software [23].

Standard PCR amplifications were carried out using NH_4SO_4 buffer, 0.1 unit/μl reaction volume Taq DNA polymerase (Fermentas, Hanover, MD, USA), 2.5 mM $MgCl_2$, 12.5 μM each dNTP, 2.5 mM each PCR amplification primer, and approximately 100 ng of template DNA. Samples were denatured for 5 min at 94°C followed by 40 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, and finally 72°C for 7 min. The annealing temperature was optimized to accommodate the respective primer.

Residual amplification primers and dNTPs were removed from the PCR product using a gel extraction kit (Millipore, Bedford, MA, USA). Amplicons were then subjected to nucleotide sequence determination and analyzed on an ABI Prism 377 sequencer (Applied Biosystems, Foster City, CA, USA).

The nucleotide sequences were initially evaluated for a sequence change between affected cats, the published normal feline sequence, and two control mixed-breed cats. A base pair change was to be considered causative for hypertrophic cardiomyopathy if it met the following criteria: was present in the affected cats but not the controls or the published feline sequence, changed a conserved amino acid, or changed the amino acid to one of a different polarity, acid/base status, or structure. The change that met the above criteria was then evaluated in 108 additional control cats.

Subsequently the amino acid substitution identified was evaluated with the PolyPhen (http://tux.embl-heidelberg.de/ramensky/polyphen.cgi) program to predict the possible impact of the amino acid substitution on the protein. PolyPhen was used to predict the likelihood that the substitution was damaging and to calculate PSIC scores, which represent the logarithmic ratio of the likelihood of a given amino acid occurring at a particular site relative to the likelihood of this amino acid occurring at any site [24]. A PSIC score difference above 2 indicates a damaging effect, between 1.5 and 2 suggests that the variant is possibly damaging, and below 0.5 indicates that the variant is benign.

Finally, the normal and altered sequences were evaluated for changes that might occur in the secondary structure with two protein structure software

programs, GOR4 (PBIL, France) and Protean (DNASTAR, Madison, WI, USA). GOR4 predicts the probability of a secondary structure at each amino acid position. Protean used the Eisenberg method to predict the hydrophobicity of the region based on computation and experimental measurements that describe the distribution of hydrophilic and hydrophobic residue groups in a protein.

A *t* test was performed to determine if there was a significant difference in the age of diagnosis between the heterozygous and the homozygous cats. A value of $p < 0.05$ was considered significant.

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